Genotypic Heterogeneity of *Orientia tsutsugamushi* in Scrub Typhus Patients and Thrombocytopenia Syndrome Co-infection, Myanmar

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Serologic and molecular surveillance of serum collected from 152 suspected scrub typhus patients in Myanmar revealed *Orientia tsutsugamushi* of genotypic heterogeneity. In addition, potential co-infection with severe fever with thrombocytopenia syndrome virus was observed in 5 (3.3%) patients. Both scrub typhus and severe fever with thrombocytopenia syndrome are endemic in Myanmar.

Scrub typhus is a miteborne febrile illness caused by the bacterium *Orientia tsutsugamushi*, which is endemic in the Asia-Pacific region and a major cause of undifferentiated febrile disease (*1*). *O. tsutsugamushi* infections were documented in Myanmar during the 1940s (*2*). Since then, however, no report has described the prevalence and genetics of scrub typhus in Myanmar, although 2 studies, including 1 from 2017, identified scrub typhus as one of the primary infections causing acute febrile illness on the Thailand–Myanmar border (*3*,*4*). These results underscore the need for research on this vectorborne infection in Myanmar, including studies defining the genotypic diversity of *O. tsutsugamushi*. Lack of this information has been a serious obstacle to developing effective diagnostic methods and a vaccine for scrub typhus (*1*).

The tickborne virus severe fever with thrombocytopenia syndrome virus (SFTSV), of the genus *Banyangvirus*, can cause hemorrhagic fever with a mortality rate of up to 40% (*5*). SFTSV infections are endemic in eastern Asia, and retrospective studies have confirmed its presence in China in 1996 (*6*), South Korea in 2000 (*7*), Japan in 2005 (*8*), and Vietnam in 2017 (*9*). In addition, mixed infection with SFTSV and *O. tsutsugamushi* has been detected in patients in South Korea, where both pathogens are endemic (*7*,*10*). These results further emphasize the urgent need for epidemiologic studies of vectorborne diseases in areas of endemicity to improve our ability to accurately differentiate febrile infectious diseases with atypical signs and symptoms during the initial stages so they can be promptly treated. Here, we used blood samples from suspected scrub typhus patients in Myanmar to investigate the serologic prevalence and genotypic diversity of *O. tsutsugamushi*. We also examined these patients for possible co-infection with SFTSV, which has been an emerging threat to public health in eastern Asia.

The Study

To investigate the genotypic diversity of *O. tsutsugamushi* and potential co-infection with SFTSV in Myanmar, we collected whole blood samples from 152 clinically suspected scrub typhus patients (Table; Appendix Table 1, https://wwwnc.cdc.gov/EID/article/26/8/20-0135-App1.pdf) in Sagaing and Magway Provinces (Figure 1) during February 2018–January 2019. Mean age of the suspected scrub typhus patients was 27 ± 19.8 years (range of 2–73 years). We observed eschar, a selection criteria for scrub typhus, in 144 (94.7%) of the 152 patients. Mean fever duration was 6 days (SD ± 2.9 days).

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Table. Baseline characteristics and summary of serologic and molecular diagnosis of suspected scrub typhus patients enrolled in study of genotypic heterogeneity of *Orientia tsutsugamushi*, Myanmar

Category	Value
Age	
Age, y mean ± SD	27.0 ± 19.8
Age distribution, y	
≤10	38 (25.0)
11–20	38 (25.0)
21–30	23 (15.1)
31–40	10 (6.6)
41–50	17 (11.0)
51–60	16 (10.5)
≥61	10 (6.6)
Sex ratio, M:F (% male)	93/59 (61.2)
Clinical variables	
Fever duration, d, mean ±SD	6.1 ± 2.9
Eschar	144 (94.7)
Rash	3 (2.0)
Myalgia	25 (20)
Method of diagnosis of scrub typhus	
ICT	41/128 (32.0)
TSA56 IgG	36/128 (28.1)
ScaA IgG	25/128 (19.5)
IFA	138/152 (90.8)
O. tsutsugamushi IgG	119/152 (78.3)
O. tsutsugamushi IgM	90/152 (59.2)
PCR (*tsa56*)	9/152 (5.9)
Method of diagnosis of SFTSV	
RT-PCR	5/152 (3.3)

*Values are no. (%) patients or no. patients/no. tested (%) except as indicated. ICT, immunochromatography test; IFA, indirect immunofluorescence assay; RT-PCR, reverse transcription PCR; SFTSV, severe fever with thrombosis syndrome virus.

For initial serologic diagnosis of 128 serum samples, we used immunochromatography test strips coated with TSA56 and ScaA antigens, which revealed respective positive rates of 28.1% (36/128) and 19.5% (25/128); the overall positive rate was 32.0% (41/128). Of the 128 samples, 20 (15.6%) reacted with both antigens and 5 of 36 (13.9%) were positive for ScaA antigen only (Table; Appendix Figure 1), suggesting a potential applicability of ScaA antigen, when used simultaneously with TSA56 antigen, for the diagnosis of scrub typhus during the acute phase (*11*). To confirm serologic positivity against the bacterial antigen, we also conducted an indirect immunofluorescence assay using cells infected with *O. tsutsugamushi*, the standard method for diagnosing scrub typhus (*12*). Among the 152 serum samples we tested, results were positive (≥1:40) for 119 (78.3%) for specific IgG and for 90 (59.2%) for IgM (Table; Appendix Table 1). Median titers of the positive serum samples were 1:640 for both IgG and IgM. Among the suspected scrub typhus patients, test results for 13 (8.6%) serum samples were negative for both IgG and IgM against *O. tsutsugamushi*.

For molecular diagnosis of scrub typhus, we examined all the serum samples by PCR to confirm infection and identify the genotypes of *O. tsutsugamushi* in the patients in Myanmar. From the 152 serum samples, we detected specific PCR products in 9 (5.9%) and sequenced them for genotyping. We compared results of phylogenetic analysis of the 9 *tsa56* gene sequences with sequences from 17 protogenotypes (*1*), which revealed ≥5 genotypes, including Karp_A (4/9, 44.4%), Karp_B (1/9, 11.1%), Kato_B (2/9, 22.2%), Gilliam (1/9, 11.1%), and JG_C (1/9, 11.1%) (Figure 2).

Figure 1. Locations in Sagaing and Magway Provinces in Myanmar, where suspected scrub typhus patients' serum samples were collected for study of genotypic heterogeneity of *Orientia tsutsugamushi*.

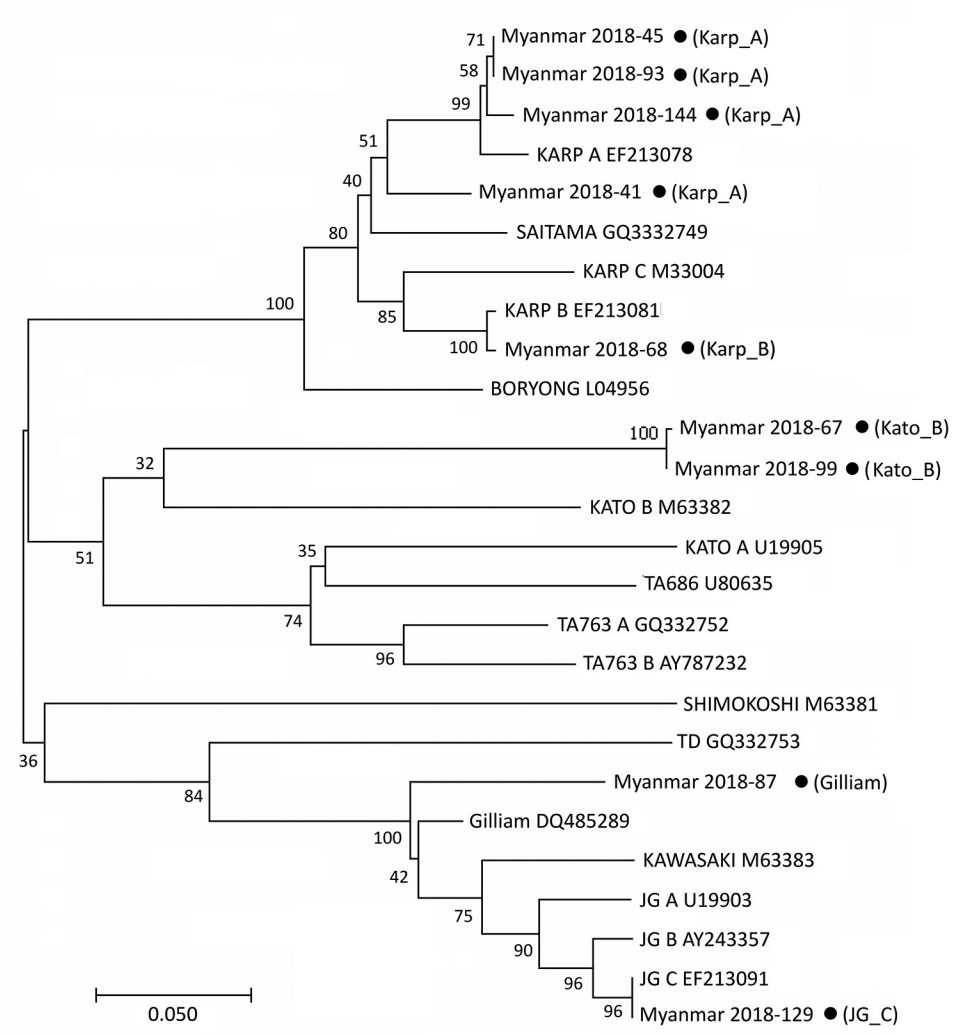

Figure 2. Phylogenetic tree constructed on the basis of *Orientia tsutsugamushi tsa56* gene sequences for scrub typhus patients in Myanmar (black dots) and reference sequences. The tree was constructed using the maximum likelihood method with MEGA7 (http://www.megasoftware.net). The *tsa56* gene sequences identified in this study are indicated by black circles and compared with 17 representative genotype sequences reported by a previous study (*1*). The percentage of replicate trees in which the associated genotypes clustered together in the bootstrap test (1,000 replicates) is shown next to the branches.

Finally, we used reverse transcription PCR analysis to investigate possible SFTSV infection in the patients (*7*,*9*,*13*). Among 152 patients' serum samples, 5 (3.3%) were positive for the partial small (S) segment of the SFTSV RNA genome, indicating SFTSV infection. Results from phylogenetic analysis of the partial S segment sequences showed that 4 isolates were the same as those previously reported from Vietnam (*9*); 1 isolate differed by 1 base from the other 4 isolates (Appendix Figure 2), suggesting genetic homogeneity of SFTSV in southern Asia. Of note, 4 out of the 5 SFTSV-positive patients had eschar, and 4 were <15 years of age (Appendix Figure 2). Furthermore, 3 of them carried high titers (≥1:2560) of IgG, IgM, or both specific to *O. tsutsugamushi*, as measured by indirect immunofluorescence assay (Appendix Table 1), suggesting co-infection with scrub typhus. All patients were successfully treated and recovered, including the SFTSV-positive febrile patients, after 5–7 days of fever.

Conclusions

We observed a high prevalence of antibodies against *O. tsutsugamushi* in suspected scrub typhus patients in Myanmar, suggesting that scrub typhus, previously reported in the 1940s, remains prevalent in this country (*2*). Of note, a high prevalence of scrub typhus in children was confirmed (*4*); therefore, young children with febrile illness should be carefully observed for early diagnosis and treatment of scrub typhus. Because we were only able to examine serum samples collected from patients during the acute phase of infection and could not assess the rise of antibody titers in paired samples collected in convalescent phases, we were not able to confirm the exact rate of prevalence of scrub typhus in the suspected patients. The baseline levels of antibody titers against *O. tsutsugamushi* in healthy persons need to be assessed to determine the cutoff titer levels for diagnosing acute scrub typhus in the endemic region (*14*).

In addition, genotyping *O. tsutsugamushi* revealed that ≥5 different genotypes are currently present and showed genetic heterogeneity in Myanmar. Moreover, we detected possible co-infection with SFTSV and *O. tsutsugamushi* in 5 patients. None of these patients had a history of travel abroad, and all live in the same village in Sagaing Province, suggesting that there may be hot spots for SFTSV infection. Co-infection with *O. tsutsugamushi* and SFTSV might be mediated by either simultaneous transmission from 2 different vectors each carrying 1 pathogen or by a single tick or mite species carrying both pathogens (*10*). Four of 5 SFTSV-positive patients were <15 years of age, and all 5 recovered within a week. Given that the disease severity of SFTS is associated with host age and the viral genotype (*15*), milder clinical symptoms observed in these patients might have been because of exposure at a younger age or prevalence of less virulent genotypes of SFTSV in Myanmar. Therefore, continuous surveillance of SFTS patients needs to be conducted, reporting detailed clinical manifestations and associated viral genotypes prevalent in the local area. In addition, more reliable differential diagnosis techniques and prevention and control measures are required for better clinical practices and outcomes in the endemic regions of multiple tickborne and miteborne pathogens.

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Appendix

Study design and ethics

To investigate the genotypic diversity of *Orientia tsutsugamushi* and potential coinfection with SFTSV in Myanmar, whole blood samples were collected from clinically suspected scrub typhus patients in Sagaing and Magway provinces during February 2018–January 2019. We selected suspected scrub typhus patients if they presented ≥3 clinical manifestations, from among fever, headache, eschar, rash, myalgia, joint pain, lymphadenopathy, and respiratory symptoms (*1*). Specimens collected from 152 patients were transported to the University of Medicine 1 (Yangon, Myanmar) to prepare serum samples, which were stored at –80°C until used for testing at Seoul National University. This study was approved by the ethics review committee of the Department of Medical Research, Ministry of Health and Sports in Myanmar (Ethics/DMR/2018/134) and the institutional review boards of Seoul National University Hospital (IRB 1910–057–1069).

Serologic test

Immunochromatography test strips containing TSA56 and ScaA antigens from *O. tsutsugamushi* of Boryong, Gilliam, and Karp genotypes were manufactured (Bore Da Biotech Co., http://boreda.com/) and used, according to the manufacturer's instructions, to test serum samples for rapid diagnosis of scrub typhus. Results were visualized in 15 min after loading mixtures of a chasing buffer (200 μL) containing gold particles and 50 μL of patient serum on a sample pad. For immunofluorescence assay, we serially diluted serum samples from 1:40 to 1:10240 in phosphate buffered saline and incubated with pooled L929 cells infected with three genotypes of *O. tsutsugamushi* (Karp, Gilliam and Boryong), as previously described (*2*).

Amplification of nucleic acids and sequence analysis

From the serum samples, we extracted total DNA, using DNeasy Blood and Tissue kit, or viral RNA, using QIAamp viral RNA Mini kit (both from QIAGEN, https://www.qiagen.com/br/), according to the manufacturer's instructions. For molecular diagnosis, we performed PCR to amplify the *tsa56* gene of *O. tsutsugamushi* using 2 sets of primers (forward primer 1: GATCAAGCTTCCTCAGCCTACTATAATGCC, reverse primer 1: CGACAGATGC ACTATTAGGC, forward primer 2: TAGTGCAATGTCTGCGTTGTCGTTGCC, reverse primer 2: ACGCTGCAATTTAACAAGATCTTTATATAACT). We sequenced the amplified DNA fragments using the same primer sets and the partial sequences of the *O. tsutsugamushi tsa56* genes were deposited in GenBank under accession nos. MN913341 to MN913349.

To detect SFTSV RNA, we performed reverse transcription PCR to amplify the partial small (S) segment of the viral RNA from the serum samples and confirm SFTSV infection (*3*). We sequenced the PCR products using the BigDye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems, https://www.perkinelmer.com/). We performed phylogenetic analysis of *O. tsutsugamushi tsa56* gene sequences and the partial S segment sequences of SFTSV using MEGA7 software (https://www.megasoftware.net/) and constructed phylogenetic trees using the maximum likelihood method (*4*).

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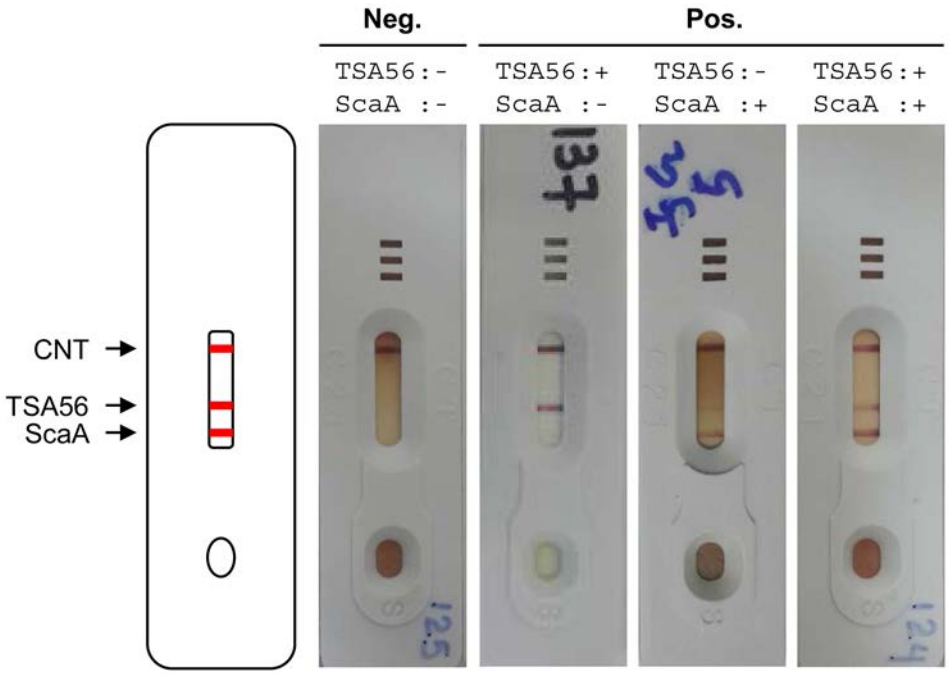
Appendix Table 1. Baseline characteristics and the results of serological and molecular diagnosis of suspected scrub typhus patients enrolled in this study

ID	Age (y)	Sex	Province	Fever duration (d)	Eschar	Muscle & joint pain	Rash	Scrub typhus IgG (ICT)		Scrub typhus IFA titer		*tsa56* PCR (genogroup)	SFTS RT-PCR
								TSA56	ScaA	IgG	IgM		
1	10	F	Sagaing	5	–	–	–	+	+	640	640	–	–
2	13	M	Sagaing	4	+	–	–	+	+	10,240	160	–	–
3	2	F	Sagaing	5	+	NA	–	–	–	2,560	80	–	–
4	7	M	Sagaing	4	+	NA	–	+	+	640	–	–	–
5	23	M	Sagaing	5	+	–	–	–	+	2,560	40	–	–
6	3	F	Sagaing	3	+	–	–	–	–	160	160	–	–
7	73	F	Sagaing	4	+	–	–	–	–	640	–	–	–
8	5	M	Sagaing	5	+	–	–	–	–	–	40	–	–
9	12	M	Sagaing	5	+	–	–	+	+	2,560	160	–	–
10	39	M	Sagaing	5	+	–	–	+	+	2,560	–	–	–
11	12	M	Sagaing	15	+	–	–	+	+	640	320	–	–
12	52	M	Sagaing	7	+	NA	NA	–	–	640	–	–	–
13	55	F	Sagaing	5	+	–	–	–	+	10,240	–	–	–
14	10	F	Sagaing	4	+	–	–	+	+	2,560	–	–	–
15	71	F	Sagaing	10	+	NA	NA	–	–	10,240	–	–	–
16	57	F	Sagaing	4	+	–	–	–	–	2,560	640	–	–
17	3	M	Sagaing	7	+	NA	NA	–	–	2,560	–	–	–
18	55	F	Sagaing	2	+	–	NA	+	+	10,240	640	–	–
19	24	F	Sagaing	10	+	+	NA	–	–	640	40	–	–
20	42	F	Sagaing	15	+	+	NA	+	–	640	640	–	–
21	42	M	Sagaing	5	+	–	–	+	+	2,560	160	–	–
22	12	F	Sagaing	4	+	–	–	+	–	640	640	–	–
23	5	F	Sagaing	3	+	+	–	–	–	2,560	160	–	–
24	51	F	Sagaing	6	+	–	–	+	+	10,240	–	–	–
25	15	M	Sagaing	10	+	–	–	–	–	2,560	160	–	–
26	20	M	Sagaing	8	+	–	–	+	+	2,560	–	–	–
27	8	M	Sagaing	3	+	–	–	–	–	2,560	2,560	–	–
28	5	M	Sagaing	3	+	–	–	–	–	160	160	–	–
29	53	M	Sagaing	4	+	–	–	–	–	640	640	–	–
30	6	M	Sagaing	7	–	–	–	–	–	–	160	–	–
31	8	M	Sagaing	3	+	–	–	–	–	40	160	–	–
32	26	F	Sagaing	5	+	–	–	–	–	160	–	–	–
33	55	M	Sagaing	5	+	–	–	–	–	–	–	–	–
34	25	M	Sagaing	7	+	–	+	–	–	160	–	–	–
35	23	F	Sagaing	7	–	+	+	+	–	160	–	–	–
36	23	F	Sagaing	5	+	–	–	+	+	10,240	10,240	–	–
37	19	F	Sagaing	8	–	–	–	–	–	160	–	–	–
38	10	M	Sagaing	3	+	–	–	–	–	–	–	–	–
39	15	M	Sagaing	3	+	–	–	–	–	–	–	–	–
40	7	M	Sagaing	7	+	–	–	–	–	–	160	–	–
41	15	M	Sagaing	7	+	+	–	–	–	–	640	+ (Karp)	–
42	37	M	Sagaing	10	–	–	–	+	+	10,240	–	–	–
43	5	M	Sagaing	3	+	–	–	–	–	–	–	–	–
44	50	M	Sagaing	6	+	+	–	–	–	160	–	–	–
45	11	M	Sagaing	10	+	–	–	–	–	5,120	640	+ (Karp)	–
46	53	F	Sagaing	5	+	–	–	–	+	10,240	–	–	–
47	13	M	Sagaing	5	+	–	–	–	–	10,240	40	–	–
48	68	F	Sagaing	3	+	–	–	–	–	10,240	–	–	–
49	72	F	Sagaing	15	+	–	–	–	–	10,240	–	–	–
50	10	M	Sagaing	4	+	–	–	–	–	10,240	40	–	–
51	16	F	Sagaing	5	+	–	–	–	–	5,120	160	–	–
52	12	M	Sagaing	7	+	–	–	+	+	10,240	2,560	–	–
53	59	M	Sagaing	8	+	–	–	–	–	10,240	–	–	–
54	25	M	Sagaing	8	+	–	–	–	–	1280	–	–	–
55	6	M	Sagaing	2	+	–	–	–	–	640	–	–	–

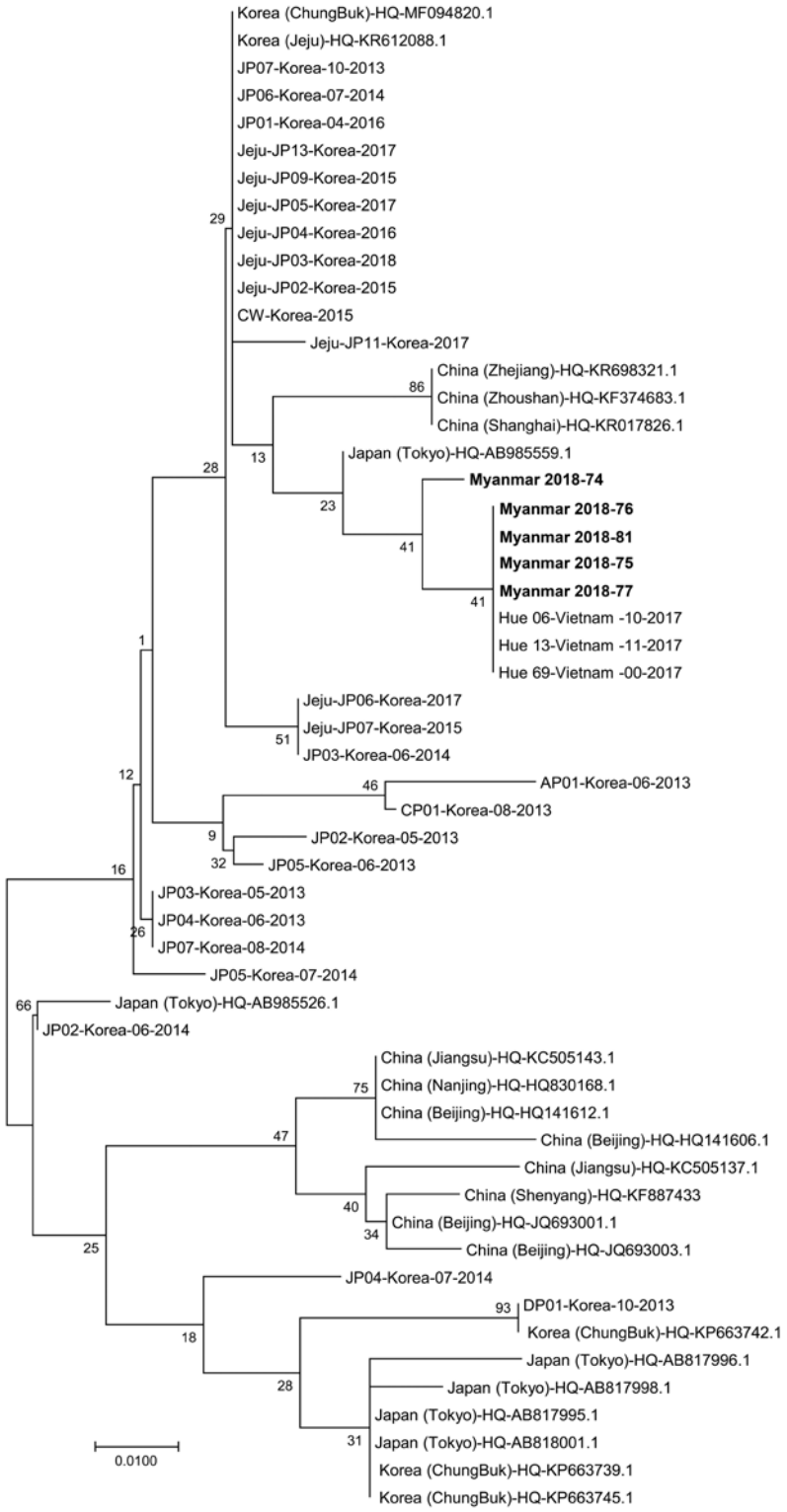
ID	Age (y)	Sex	Province	Fever duration (d)	Eschar	Muscle & joint pain	Rash	Scrub typhus IgG (ICT)		Scrub typhus IFA titer		*tsa56* PCR (genogroup)	SFTS RT-PCR
								TSA56	ScaA	IgG	IgM		
56	67	M	Sagaing	20	+	–	–	–	–	10,240	640	–	–
57	13	F	Sagaing	3	+	–	–	–	–	320	–	–	–
58	11	F	Sagaing	3	+	–	–	–	–	2,560	–	–	–
59	20	M	Sagaing	5	+	–	–	–	–	10,240	–	–	–
60	13	M	Sagaing	5	+	–	–	–	–	10,240	640	–	–
61	10	F	Sagaing	8	+	–	–	–	–	10,240	10,240	–	–
62	20	M	Sagaing	5	+	–	–	–	–	10,240	–	–	–
63	38	F	Sagaing	4	+	–	+	–	–	5125	–	–	–
64	3	M	Sagaing	2	+	–	–	–	–	80	–	–	–
65	76	M	Sagaing	10	+	–	–	–	–	320	–	–	–
66	26	F	Sagaing	3	+	+	–	–	–	640	160	–	–
67	10	M	Sagaing	5	+	–	–	–	–	320	160	+ (Kato)	–
68	16	F	Sagaing	5	+	–	–	–	–	640	160	+ (Karp)	–
69	30	M	Sagaing	4	+	–	–	–	–	160	–	–	–
70	12	M	Sagaing	4	+	–	–	–	+	2,560	–	–	–
71	17	M	Sagaing	3	+	–	–	–	–	–	–	–	–
72	35	M	Sagaing	3	+	–	–	–	–	10,240	–	–	–
73	14	M	Sagaing	5	+	–	–	–	–	640	–	–	–
74	10	M	Sagaing	5	+	–	–	–	+	10,240	–	–	+
75	4	M	Sagaing	5	+	–	–	NT		160	40	–	+
76	6	M	Sagaing	5	+	–	–	NT		2,560	2,560	–	+
77	42	M	Sagaing	7	–	–	–	NT		10,240	–	–	+
78	7	M	Sagaing	7	+	–	–	NT		10,240	640	–	–
79	59	F	Sagaing	5	+	+	–	NT		10,240	–	–	–
80	40	M	Sagaing	5	+	–	–	NT		–	160	–	–
81	14	M	Sagaing	5	+	–	–	NT		–	40	–	+
82	5	M	Sagaing	5	+	–	–	NT		2,560	10,240	–	–
83	60	F	Sagaing	5	–	–	–	NT		2,560	–	–	–
84	16	M	Sagaing	5	+	–	–	NT		640	2,560	–	–
85	9	M	Sagaing	5	+	–	–	NT		2,560	2,560	–	–
86	20	F	Sagaing	10	+	–	–	NT		2,560	10,240	–	–
87	10	M	Sagaing	3	+	–	–	NT		2,560	2,560	+ (Gilliam)	–
88	50	F	Sagaing	7	+	–	–	NT		5,120	–	–	–
89	41	M	Sagaing	5	+	–	–	NT		160	–	–	–
90	13	M	Sagaing	3	+	–	–	NT		–	–	–	–
91	11	M	Sagaing	5	+	–	–	NT		40	–	–	–
92	10	M	Sagaing	4	+	–	–	NT		160	–	–	–
93	29	M	Sagaing	5	+	–	–	NT		–	–	+ (Karp)	–
94	10	M	Sagaing	5	+	–	–	NT		160	–	–	–
95	5	F	Sagaing	NA	+	–	–	NT		–	–	–	–
96	63	F	Sagaing	5	+	–	–	NT		–	–	–	–
97	5	F	Sagaing	5	+	–	–	NT		640	2,560	–	–
98	9	M	Sagaing	5	+	–	–	NT		640	10,240	–	–
99	10	M	Sagaing	5	+	–	–	–	–	–	40	+ (Kato)	–
100	12	M	Sagaing	4	+	–	–	+	+	10,240	10,240	–	–
101	12	F	Sagaing	10	+	–	–	+	–	2,560	2,560	–	–
102	50	M	Sagaing	5	+	–	–	+	–	640	–	–	–
103	45	F	Sagaing	5	+	–	–	–	–	40	–	–	–
104	40	F	Sagaing	6	+	NA	–	–	–	40	40	–	–
105	26	M	Sagaing	3	+	–	–	–	–	160	–	–	–
106	42	M	Sagaing	10	+	–	–	+	–	2,560	–	–	–
107	12	F	Sagaing	5	+	–	–	–	–	160	160	–	–
108	19	F	Sagaing	3	+	–	–	+	–	640	640	–	–
109	10	F	Sagaing	4	+	–	–	–	–	–	80	–	–
110	36	M	Sagaing	2	+	–	–	–	–	–	40	–	–
111	15	M	Sagaing	7	+	–	–	–	–	–	–	–	–
112	19	M	Sagaing	7	+	+	–	–	–	–	2,560	–	–
113	12	M	Sagaing	7	+	–	–	–	–	–	10,240	–	–
114	21	M	Sagaing	5	+	–	–	+	–	640	160	–	–
115	11	F	Sagaing	3	+	–	–	–	–	40	2,560	–	–
116	29	M	Sagaing	5	+	+	–	–	–	40	640	–	–
117	33	M	Sagaing	5	+	–	–	–	–	–	80	–	–
118	11	M	Sagaing	7	–	–	–	+	+	640	2,560	–	–
119	6	F	Sagaing	3	+	–	–	+	+	160	160	–	–
120	21	M	Sagaing	5	+	–	–	–	–	–	–	–	–
121	8	F	Sagaing	5	+	–	–	+	–	80	10,240	–	–
122	54	M	Sagaing	7	+	–	–	+	+	160	640	–	–

ID	Age (y)	Sex	Province	Fever duration (d)	Eschar	Muscle & joint pain	Rash	Scrub typhus IgG (ICT)		Scrub typhus IFA titer		*tsa56* PCR (genogroup)	SFTS RT-PCR
								TSA56	ScaA	IgG	IgM		
123	66	M	Sagaing	10	+	–	–	+	+	640	–	–	–
124	62	F	Sagaing	12	+	–	–	+	+	640	1280	–	–
125	43	F	Sagaing	7	+	–	–	–	–	–	–	–	–
126	29	M	Sagaing	4	+	–	–	+	–	80	–	–	–
127	8	M	Sagaing	3	+	–	–	–	–	40	160	–	–
128	14	M	Sagaing	5	–	–	–	–	–	40	640	–	–
129	21	F	Sagaing	5	+	–	–	–	–	640	2,560	+ (Gilliam)	–
130	25	F	Magway	7	+	–	–	–	–	–	640	–	–
131	11	F	Magway	5	+	–	–	–	–	160	10,240	–	–
132	33	F	Magway	8	+	+	–	–	–	–	40	–	–
133	44	F	Magway	7	+	–	–	–	–	–	–	–	–
134	52	F	Magway	9	+	–	–	–	–	–	40	–	–
135	50	F	Magway	10	+	+	–	–	–	640	10,240	–	–
136	50	F	Magway	9	+	+	–	–	–	2,560	640	–	–
137	45	M	Magway	5	+	+	–	+	–	10,240	2,560	–	–
138	62	M	Magway	10	+	–	–	+	–	10,240	10,240	–	–
139	24	M	Magway	7	+	+	–	–	–	–	10,240	–	–
140	40	F	Magway	10	+	+	–	–	–	40	80	–	–
141	50	F	Magway	10	+	+	–	+	–	–	2,560	–	–
142	44	F	Magway	10	+	+	–	–	–	640	10,240	–	–
143	30	M	Magway	8	+	–	–	–	–	640	10,240	–	–
144	26	M	Magway	10	+	+	–	–	–	160	640	+ (Karp)	–
145	53	F	Magway	7	+	+	–	–	–	–	640	–	–
146	55	M	Magway	15	+	+	–	+	–	640	10,240	–	–
147	23	M	Magway	6	+	+	–	–	–	80	640	–	–
148	22	M	Magway	6	+	+	–	+	–	–	–	–	–
149	30	M	Magway	10	+	–	–	–	–	–	40	–	–
150	57	M	Magway	12	+	+	–	–	–	40	10,240	–	–
151	46	M	Magway	7	+	+	–	+	–	160	2,560	–	–
152	5	F	Magway	7	+	–	–	–	–	40	10,240	–	–

ICT, immunochromatography test; IFA, immunofluorescence assay; NA, not available; NT, not tested; RT, reverse transcription; SFTS, severe fever with thrombosis syndrome; +, positive result; –, negative result



Appendix Figure 1. Representative images of ICT results detecting specific IgG against TSA56 and ScaA antigen in suspected scrub typhus patients' sera.



Appendix Figure 2. Phylogenetic tree constructed based on partial S segment sequences of SFTSV. The tree was constructed using the maximum likelihood method with MEGA7. The partial S segment sequences amplified from the serum samples of indicated patients were analyzed and are shown in red.